

## IX. ABSTRACT

Beta-amyloid peptide ( $\beta$ A) is a major fibrillar component of neuritic plaques in Alzheimer's disease brains and is related to the pathogenesis of the disease.  $\beta$ A generation depends on proteolytic cleavage of the amyloid precursor protein (APP).

5        The present invention is a new procedure for the cloning of human  $\beta$ A precursor protein gene (human APP gene) based on the reverse transcription (RT) and the polymerase chain reaction (PCR). This procedure for cloning human APP gene by means of RT-PCR reactions is cost-effective, not time-consuming, and is suited for any laboratory.

10        The present invention also includes a new procedure for the construction of expression plasmids, a/ using the pFastBac<sup>TM</sup> HTb and the pBlueBacHis2 A transfer vectors for the purpose of obtaining human APP in insect cells; and b/ using the pET-28a (+) transfer vector for the purpose of obtaining human APP in bacteria.

15        The present invention makes it easier to obtain full-length APP which is essential for the identification of biological activities that occur in the APP molecule and for the identification of proteases capable of creating  $\beta$ A. Knowing which protease creates  $\beta$ A is important for the exploration of therapeutic and preventative strategies for the treatment of Alzheimer's disease.

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## LEGEND OF FIGURES

**Figure 1:** PCR amplification of the human APP gene.

M: Marker.

1: PCR product of the human APP gene (~ 2.3 kb).

5 **Figure 2:** Cloning of the APP-cDNA into pCR<sup>R</sup> II plasmid vector.

The arrow head point is the direction of transcription.

(1): pCR<sup>R</sup> II/APP<sub>751</sub>-cDNA.

(2): pCR<sup>R</sup> II/APP<sub>770</sub>-cDNA.

**Figure 3:** Construction of pFastBac<sup>TM</sup> HTb/APP-cDNA.

10 The arrow head point is the direction of transcription.

(3): pFastBac<sup>TM</sup> HTb/APP<sub>751</sub>-cDNA.

(4): pFastBac<sup>TM</sup> HTb/APP<sub>770</sub>-cDNA.

(5): APP<sub>751</sub>-cDNA in bacmid of DH10Bac<sup>TM</sup> E. Coli.

(6): APP<sub>770</sub>-cDNA in bacmid of DH10Bac<sup>TM</sup> E. Coli.

15 **Figure 4:** Construction of pBlueBacHis2 A/APP-cDNA.

The arrow head point is the direction of transcription.

(7): pBlueBacHis2 A/APP<sub>751</sub>-cDNA.

(8): pBlueBacHis2A/APP<sub>770</sub>-cDNA.

**Figure 5:** Construction of pET-28a (+)/APP-cDNA.

20 The arrow head point is the direction of transcription.

(9): pET-28a (+)/APP<sub>751</sub>-cDNA.

(10): pET-28 (+)/APP<sub>770</sub>-cDNA.